encoding an amplifiable marker, wherein said vector construct does not comprise a homologous targeting sequence.

59. (Once amended) A vector construct comprising a transcriptional regulatory sequence, a sequence encoding an amplifiable marker, and a viral origin of replication, wherein said vector construct does not comprise a homologous targeting sequence.

73. (Once amended) The cell of claim 71, wherein an endogenous gene is over-expressed in said cell by upregulation of the gene by said transcriptional regulatory sequence on said vector construct.

77. (Twice amended) A method for producing an expression product of an endogenous gene or portion thereof comprising:

(a) introducing the vector construct of either claim 58 or 59 into a eukaryotic cell;

(b) integrating said vector construct into the genome of said cell by non-homologous recombination; and

(c) over-expressing said endogenous gene in said cell.

81. (Twice amended) A cell library comprising a collection of eukaryotic cells transformed with the vector construct of claim 58 or 59, wherein said vector construct is integrated into the genomes of said cells by non-homologous recombination.

85. (Once amended) A method for over-expressing an endogenous gene in a cell *in vivo*, comprising:

(a) introducing a vector comprising a transcriptional regulatory sequence into a eukaryotic cell *in vitro*;

(b) integrating said vector into the genome of said cell by non-homologous recombination;

(c) over-expressing an endogenous gene or a portion thereof in said cell by upregulation of said endogenous gene by said transcriptional regulatory sequence;

(d) screening said cell for over-expression of said endogenous gene; and

(e) introducing said isolated and cloned cell into an animal under conditions favoring the overexpression of said endogenous gene by said cell *in vivo*.

86. (Once amended) A method for producing an expression product of an endogenous gene *in vivo*, comprising:

(a) introducing a vector comprising a transcriptional regulatory sequence operably linked to an unpaired splice donor sequence into a eukaryotic cell *in vitro*;

(b) integrating said vector integrate into the genome of said cell by non-homologous recombination;

(c) over-expressing an endogenous gene or a portion thereof in said cell by upregulation of said endogenous gene by said transcriptional regulatory sequence;

(d) screening said cell for over-expression of said endogenous gene; and

(e) introducing said isolated and cloned cell into an animal under conditions favoring the overexpression of said endogenous gene by said cell *in vivo*.

87. (Once amended) A method for producing an expression product of an endogenous gene, comprising:

(a) introducing a vector comprising a transcriptional regulatory sequence and one or more amplifiable markers into a eukaryotic cell;

(b) integrating said vector into the genome of said cell by non-homologous recombination;

(c) over-expressing an endogenous gene or a portion thereof in said cell by upregulation of said endogenous gene by said transcriptional regulatory sequence;

(d) screening said cell for over-expression of said endogenous gene;

(e) culturing said cell under conditions in which said vector and said endogenous gene are amplified in said cell; and

(f) culturing said cell under conditions favoring the production of the expression product of said endogenous gene by said cell.

89. (Twice amended) The method of claim 87, wherein said vector further comprises a splice donor site operably linked to said transcriptional regulatory sequence.

98. (Once amended) A method for over-expressing an endogenous gene in a cell *in vivo*, comprising:

(a) introducing a vector comprising a transcriptional regulatory sequence and one or more amplifiable markers into a eukaryotic cell *in vitro*;

(b) integrating said vector into the genome of said cell by non-homologous recombination;

(c) over-expressing an endogenous gene or a portion thereof in said cell by upregulation of said gene by said transcriptional regulatory sequence;

(d) screening said cell for over-expression of said endogenous gene; and

(e) introducing said isolated and cloned cell into an animal under conditions favoring the overexpression of said endogenous gene by said cell *in vivo*.

109. (Once amended) A method for producing an expression product of an endogenous gene in a cell comprising:

(a) introducing a vector comprising a transcriptional regulatory sequence into at least one isolated genome-containing eukaryotic cell;

(b) integrating said vector into the genome of said cell by non-homologous recombination;

(c) over-expressing an endogenous gene or a portion thereof in said cell by upregulation of said endogenous gene by said transcriptional regulatory sequence;

(d) screening said cell for over-expression of said endogenous gene; and

(e) culturing said cell in reduced serum medium.

110. (Once amended) A method of protein discovery comprising:

(a) introducing a vector comprising a transcriptional regulatory sequence into at least one isolated genome-containing eukaryotic cell;

(b) integrating said vector into the genome of said cell by non-homologous recombination;

(c) culturing said cell in reduced serum medium under conditions that allow over-expression of an endogenous gene or a portion thereof in said cell by upregulation of said endogenous gene by said transcriptional regulatory sequence, thereby producing cell-conditioned media; and

(d) screening said cell-conditioned media for the presence of the expression product of said gene or portion thereof.

113. (Once amended) A method for producing an expression product of an endogenous gene comprising:

(a) introducing a vector comprising a transcriptional regulatory sequence into a eukaryotic cell;

(b) integrating said vector into the genome of said cell by non-homologous recombination;

(c) over-expressing said endogenous cellular gene or a portion thereof in said cell by upregulation of said gene by said transcriptional regulatory sequence;

(d) screening said cell for over-expression of said gene;

(e) culturing said cell under conditions favoring the production of the expression product of said gene by said cell; and

(f) isolating said expression product from a cell mass equivalent to at least 10 liters of cells at 10^4 cells/ml.

116. (Once amended) A method for increasing expression of an endogenous gene in a cell *in situ*, the phenotype of said endogenous gene being known, without making use of any sequence information of the gene, the method comprising the steps of:

(a) constructing a vector comprising an amplifiable marker, a transcriptional regulatory sequence, and an unpaired splice donor sequence;

(b) delivering copies of the vector to a plurality of eukaryotic cells;

(c) culturing the cells under conditions permitting nonhomologous recombination events between the inserted vector and the genome of the cells;

(d) screening the non-homologously recombinant cells by assay for the phenotype of said endogenous gene to identify cells in which the expression of said gene has been enhanced; and

(e) selecting for cells with increased expression of said amplifiable marker and said endogenous gene.

118. (Twice amended) An isolated eukaryotic cell comprising in its genome an inserted genetic construct, said genetic construct comprising an amplifiable marker and a transcriptional regulatory sequence, wherein said genetic construct is inserted into a gene or an

upstream region of a gene and activates the expression of said gene, and wherein said gene and upstream region of said gene have no nucleotide sequence homology required for said insertion to said genetic construct.

157. (Once amended) A vector construct comprising:

(a) a first promoter operably linked to a sequence encoding a positive selectable marker;

(b) a second promoter operably linked to a sequence encoding a negative selectable marker; and

(c) an unpaired splice donor site,

wherein said positive and negative selectable markers and said splice donor site are oriented in said vector construct in an orientation that, when said vector construct is integrated into the genome of a eukaryotic host cell in such a way that an endogenous gene in said genome is transcriptionally activated, then said positive selectable marker is expressed in active form and said negative selectable marker is either not expressed or is expressed in inactive form.

159. (Twice amended) The vector construct of any one of claims 58, 59, and 157, said vector further comprising one or more transposition signals.

161. (Twice amended) The vector construct of any one of claims 58, 59, and 157, said vector further comprising one or more viral origins of replication.

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162. (Twice amended) The vector construct of any one of claims 58, 59, and 157, said vector further comprising one or more viral replication factor genes.

165. (Twice amended) The vector construct of any one of claims 58, 59, and 157, said vector further comprising genomic DNA.

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166. (Twice amended) A eukaryotic host cell comprising the vector construct of any one of claims 58, 59, and 157.

167. (Once amended) A eukaryotic host cell comprising the vector construct of claim 159.

169. (Once amended) A eukaryotic host cell comprising the vector construct of claim 161.

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170. (Once amended) A eukaryotic host cell comprising the vector construct of claim 162.

171. (Once amended) A eukaryotic host cell comprising the vector construct of claim 165.

174. (Twice amended) A library of eukaryotic cells comprising the vector construct of any one of claims 58, 59, and 157.

175. (Once amended) A library of eukaryotic cells comprising the vector construct of claim 159.

177. (Once amended) A library of eukaryotic cells comprising the vector construct of claim 161.

178. (Once amended) A library of eukaryotic cells comprising the vector construct of claim 162.

179. (Once amended) A library of eukaryotic cells comprising the vector construct of claim 165.

180. (Twice amended) A method for activation of an endogenous gene in a cell comprising:

(a) transfecting a genome-containing eukaryotic cell with the vector of any one of claims 58, 59, and 157; and

(b) culturing said cell under conditions suitable for non-homologous integration of said vector into the genome of said cell, wherein said integration results in the activation of said endogenous gene in the genome of said cell.

181. (Twice amended) A method for identifying a gene comprising:

(a) transfecting a plurality of genome-containing eukaryotic cells with the vector of any one of claims 58, 59, and 157;

(b) culturing said cells under conditions suitable for non-homologous integration of the vector into the genome of said cells;

(c) selecting for cells in which said vector has integrated into the genomes of said cells to produce selected cells;

(d) isolating RNA from said selected cells;

(e) producing cDNA from said isolated RNA; and

(f) identifying a gene in said cDNA by isolating one or more cDNA molecules containing one or more nucleotide sequences from said vector.

232. (Once amended) A method for producing an expression product of an endogenous gene, comprising:

(a) introducing a vector comprising a transcriptional regulatory sequence and one or more amplifiable markers into a eukaryotic cell;

(b) integrating said vector into the genome of said cell by non-homologous recombination;

(c) over-expressing said endogenous gene or a portion thereof in said cell by upregulation of said gene by said transcriptional regulatory sequence;

(d) screening said cell for over-expression of said endogenous gene;

(e) culturing said cell under conditions in which said vector and said endogenous gene are amplified in said cell; and

(f) introducing said cell into an animal under conditions favoring the overexpression of said endogenous gene by said cell *in vivo*.

234. (Once amended) A vector construct comprising a transcriptional regulatory sequence operably linked to an unpaired splice donor sequence wherein said vector construct does not contain a poly-adenylation site operably linked to said transcriptional regulatory sequence, wherein said vector construct does not contain a targeting sequence, and wherein there is no selectable marker between the transcriptional regulatory sequence and the splice donor sequence, wherein said transcriptional regulatory sequence is selected from the group consisting of a cellular transcriptional regulatory sequence and a eukaryotic viral transcriptional regulatory sequence.

235. (Once amended) A vector construct comprising a promoter operably linked to an unpaired splice donor sequence wherein said vector construct does not contain a poly-adenylation site operably linked to said promoter, wherein said vector construct does not contain a targeting sequence, and wherein there is no selectable marker between the transcriptional regulatory sequence and the splice donor sequence, wherein said promoter is selected from the group consisting of a eukaryotic viral promoter and a cellular promoter.

236. (Once amended) A vector construct comprising a transcriptional regulatory sequence operably linked to an exon, said exon defined at the 3' end by an unpaired splice donor sequence, wherein said vector construct does not contain a poly-adenylation site operably linked to said transcriptional regulatory sequence, wherein said vector construct does not contain a targeting sequence, and wherein said exon does not contain a selectable marker coding sequence, wherein said transcriptional regulatory sequence is selected from the group consisting of a cellular transcriptional regulatory sequence and a eukaryotic viral transcriptional regulatory sequence.

237. (Once amended) A vector construct comprising a promoter operably linked to an exon, said exon defined at the 3' end by an unpaired splice donor sequence, wherein said vector construct does not contain a poly-adenylation site operably linked to said promoter, wherein said vector construct does not contain a targeting sequence, and wherein said exon does not contain a selectable marker, wherein said promoter is selected from the group consisting of a eukaryotic viral promoter and a cellular promoter.

238. (Once amended) A vector construct comprising a transcriptional regulatory sequence operably linked to an unpaired splice donor sequence wherein said vector construct does not contain a poly-adenylation site operably linked to said transcriptional regulatory sequence, wherein said vector construct does not contain a targeting sequence, and wherein there is no internal ribosome entry site between the transcriptional regulatory sequence and the splice donor sequence, wherein said transcriptional regulatory sequence is selected from the group

consisting of a cellular transcriptional regulatory sequence and a eukaryotic viral transcriptional regulatory sequence.

239. (Once amended) A vector construct comprising a promoter operably linked to an unpaired splice donor sequence wherein said vector construct does not contain a poly-adenylation site operably linked to said promoter, wherein said vector construct does not contain a targeting sequence, and wherein there is no internal ribosome entry site between the transcriptional regulatory sequence and the splice donor sequence wherein, said promoter is selected from the group consisting of a eukaryotic viral promoter and a cellular promoter.

240. (Once amended) A vector construct comprising a transcriptional regulatory sequence operably linked to an exon, said exon defined at the 3' end by an unpaired splice donor sequence, wherein said vector construct does not contain a poly-adenylation site operably linked to said transcriptional regulatory sequence, wherein said vector construct does not contain a targeting sequence, and wherein said exon does not contain an internal ribosome entry site, wherein said transcriptional regulatory sequence is selected from the group consisting of a cellular transcriptional regulatory sequence and a eukaryotic viral transcriptional regulatory sequence.

241. (Once amended) A vector construct comprising a promoter operably linked to an exon, said exon defined at the 3' and by an unpaired splice donor sequence, wherein said vector construct does not contain a poly-adenylation site operably linked to said promoter,

wherein said vector construct does not contain a targeting sequence, and wherein said exon does not contain an internal ribosome entry site, wherein said promoter is selected from the group consisting of a eukaryotic viral promoter and a cellular promoter.

243. (Once amended) A vector construct comprising a promoter operably linked to an exon defined at the 3' end by an unpaired splice donor sequence, wherein said vector construct does not contain a poly-adenylation site operably linked to said promoter, wherein said vector construct does not contain a targeting sequence, wherein said exon is derived from a naturally-occurring eukaryotic sequence, does not encode antibiotic resistance activity, and is not a reporter gene, and wherein said splice donor sequence is derived from a naturally-occurring eukaryotic splice donor sequence, wherein said promoter is selected from the group consisting of a eukaryotic viral promoter and a cellular promoter.

244. (Once amended) A vector construct comprising a promoter operably linked to an exon defined at the 3' end by an unpaired splice donor sequence, wherein said vector construct does not contain a poly-adenylation site operably linked to said promoter, wherein said vector construct does not contain a targeting sequence, wherein said exon is derived from a naturally-occurring eukaryotic sequence, does not encode antibiotic resistance activity, and is not a reporter gene, and wherein said splice donor sequence is derived from a naturally-occurring eukaryotic splice donor sequence, said vector construct further comprising a marker sequence operably linked to a promoter other than the promoter operably linked to said exon.

245. (Once amended) A retrovirus vector construct comprising a first and second retrovirus long terminal repeat sequence, a eukaryotic promoter operably linked to an exon, said exon defined at the 3' end by an unpaired splice donor sequence, wherein said exon is derived from a naturally-occurring eukaryotic sequence, does not encode antibiotic resistance activity, or is not a reporter gene, wherein said splice donor sequence is derived from a naturally-occurring eukaryotic gene, and wherein the vector construct does not contain a poly-adenylation site operably linked to said promoter.

246. (Once amended) A retrovirus vector construct comprising a first and second retrovirus long terminal repeat sequence, a eukaryotic promoter operably linked to an exon, said exon defined at the 3' end by an unpaired splice donor sequence, wherein said exon is derived from a naturally-occurring eukaryotic sequence, does not encode antibiotic resistance activity, and is not a reporter gene, wherein said splice donor sequence is derived from a naturally-occurring eukaryotic gene, and wherein the vector construct does not contain a poly-adenylation site operably linked to said promoter, wherein the promoter, exon, and splice donor sequence are present in the vector construct between the long terminal repeat sequences in opposite orientation to the long terminal repeat sequences.

247. (Once amended) A vector construct comprising a promoter operably linked to an exon defined at the 3' end by an unpaired splice donor sequence, wherein said vector construct does not contain a poly-adenylation site operably linked to said promoter, wherein said vector construct does not contain a targeting sequence, and wherein said exon is derived from a

naturally-occurring eukaryotic sequence, does not encode antibiotic resistance activity, and is not a reporter gene, wherein said promoter is selected from the group consisting of a eukaryotic viral promoter and a cellular promoter.

248. (Once amended) A vector construct comprising a promoter operably linked to an exon defined at the 3' end by an unpaired splice donor sequence, wherein said vector construct does not contain a poly-adenylation site operably linked to said promoter, wherein said vector construct does not contain a targeting sequence, and wherein said exon is derived from a naturally-occurring eukaryotic sequence, does not encode antibiotic resistance activity, and is not a reporter gene, said vector construct further comprising a marker sequence operably linked to a promoter other than the promoter operably linked to said exon.

249. (Once amended) A retrovirus vector construct comprising a first and second retrovirus long terminal repeat sequence, a eukaryotic promoter operably linked to an exon, said exon defined at the 3' end by an unpaired splice donor sequence, wherein said exon is derived from a naturally-occurring eukaryotic sequence, does not encode antibiotic resistance activity, or is not a reporter gene, and wherein the vector construct does not contain a poly-adenylation site operably linked to said promoter.

250. (Once amended) A retrovirus vector construct comprising a first and second retrovirus long terminal repeat sequence, a eukaryotic promoter operably linked to an exon, said exon defined at the 3' end by an unpaired splice donor sequence, wherein said exon is derived

from a naturally-occurring eukaryotic sequence, does not encode antibiotic resistance activity, and is not a reporter gene, wherein the vector construct does not contain a poly-adenylation site operably linked to said promoter, and wherein the promoter, exon, and splice donor sequence are present in the vector construct between the long terminal repeat sequences in opposite orientation to the long terminal repeat sequences.

253. (Once amended) A method of generating a library of eukaryotic cells comprising introducing a vector construct according to any one of claims 234–241 and 243–250 into eukaryotic cells to produce said library.

254. (Once amended) A method of generating a library of eukaryotic cells comprising introducing a vector construct according to claim 242 into eukaryotic cells to produce said library.

257. (Once amended) A method to activate expression of a gene in an isolated eukaryotic cell comprising introducing a vector construct into said cell, wherein said construct comprises a promoter operably linked to an exon defined at the 3' end by an unpaired splice donor sequence, wherein said vector construct does not contain a targeting sequence, wherein said exon is derived from a naturally-occurring eukaryotic sequence, does not encode antibiotic resistance activity, and is not a reporter gene, wherein said splice donor sequence is derived from a naturally-occurring eukaryotic splice donor sequence, wherein the vector construct is incorporated into the genome of said eukaryotic cell by non-homologous recombination and

wherein said splice donor sequence is spliced to a splice acceptor sequence in said activated gene in said isolated eukaryotic cell.

258. (Once amended) A method to activate expression of a gene in an isolated eukaryotic cell comprising introducing a vector construct into said cell, wherein said construct comprises a promoter operably linked to an exon defined at the 3' end by an unpaired splice donor sequence, wherein said vector construct does not contain a targeting sequence, wherein said exon is derived from a naturally-occurring eukaryotic sequence, does not encode antibiotic resistance activity, and is not a reporter gene, wherein the vector construct is incorporated into the genome of said eukaryotic cell by non-homologous recombination and wherein said splice donor sequence is spliced to a splice acceptor sequence in said activated gene in said isolated eukaryotic cell.

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259. (Once amended) The method of claim 257 wherein said vector construct is a retrovirus vector construct.

Please add the following new claims:

260. (New) The method of claim 232 wherein said vector further comprises a splice donor site operably linked to said transcriptional regulatory sequence.

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261. (New) A method for making a eukaryotic host cell with a vector construct integrated into the genome of said cell, the vector construct comprising a transcriptional regulatory sequence operably linked to an unpaired splice donor sequence wherein said construct does not contain a poly-adenylation site operably linked to said transcriptional regulatory sequence, wherein said vector construct does not contain a targeting sequence, and wherein there is no selectable marker between the transcriptional regulatory sequence and the splice donor sequence, said method comprising introducing said vector construct into said host cell under conditions such that said vector construct is integrated into the genome of said host cell.

262. (New) A method for making a eukaryotic host cell with a vector construct integrated into the genome of said cell, the vector construct comprising a promoter operably linked to an unpaired splice donor sequence wherein said construct does not contain a poly-adenylation site operably linked to said promoter, wherein said construct does not contain a targeting sequence, and wherein there is no selectable marker between the transcriptional regulatory sequence and the splice donor sequence, said method comprising introducing said vector construct into said host cell under conditions such that said vector construct is integrated into the genome of said host cell.

263. (New) A method for making a eukaryotic host cell with a vector construct integrated into the genome of said cell, the vector construct comprising a transcriptional regulatory sequence operably linked to an exon, said exon defined at the 3' end by an unpaired splice donor sequence, wherein said construct does not contain a poly-adenylation site operably

linked to said transcriptional regulatory sequence, wherein said construct does not contain a targeting sequence, and wherein said exon does not contain a selectable marker, said method comprising introducing said vector construct into said host cell under conditions such that said vector construct is integrated into the genome of said host cell.

264. (New) A method for making a eukaryotic host cell with a vector construct integrated into the genome of said cell, the vector construct comprising a promoter operably linked to an exon, said exon defined at the 3' and by an unpaired splice donor sequence, wherein said construct does not contain a poly-adenylation site operably linked to said promoter, wherein said construct does not contain a targeting sequence, and wherein said exon does not contain a selectable marker, said method comprising introducing said vector construct into said host cell under conditions such that said vector construct is integrated into the genome of said host cell.

265. (New) A method for making a eukaryotic host cell with a vector construct integrated into the genome of said cell, the vector construct comprising a transcriptional regulatory sequence operably linked to an unpaired splice donor sequence wherein said construct does not contain a poly-adenylation site operably linked to said transcriptional regulatory sequence, wherein said construct does not contain a targeting sequence, and wherein there is no internal ribosome entry site between the transcriptional regulatory sequence and the splice donor sequence, said method comprising introducing said vector construct into said host cell under conditions such that said vector construct is integrated into the genome of said host cell.

266. (New) A method for making a eukaryotic host cell with a vector construct integrated into the genome of said cell, the vector construct comprising a promoter operably linked to an unpaired splice donor sequence wherein said construct does not contain a poly-adenylation site operably linked to said promoter, wherein said construct does not contain a targeting sequence, and wherein there is no internal ribosome entry site between the transcriptional regulatory sequence and the splice donor sequence, said method comprising introducing said vector construct into said host cell under conditions such that said vector construct is integrated into the genome of said host cell.

267. (New) A method for making a eukaryotic host cell with a vector construct integrated into the genome of said cell, the vector construct comprising a transcriptional regulatory sequence operably linked to an exon, said exon defined at the 3' end by an unpaired splice donor sequence, wherein said construct does not contain a poly-adenylation site operably linked to said transcriptional regulatory sequence, wherein said construct does not contain a targeting sequence, and wherein said exon does not contain an internal ribosome entry site, said method comprising introducing said vector construct into said host cell under conditions such that said vector construct is integrated into the genome of said host cell.

268. (New) A method for making a eukaryotic host cell with a vector construct integrated into the genome of said cell, the vector construct comprising a promoter operably linked to an exon, said exon defined at the 3' and by an unpaired splice donor sequence, wherein said construct does not contain a poly-adenylation site operably linked to said promoter, wherein

said construct does not contain a targeting sequence, and wherein said exon does not contain an internal ribosome entry site, said method comprising introducing said vector construct into said host cell under conditions such that said vector construct is integrated into the genome of said host cell.

269. (New) A method for making a eukaryotic host cell with a vector construct integrated into the genome of said cell, the vector construct comprising a promoter operably linked to an exon defined at the 3' end by an unpaired splice donor sequence, wherein said construct does not contain a poly-adenylation site operably linked to said promoter, wherein said construct does not contain a targeting sequence, wherein said exon is derived from a naturally-occurring eukaryotic sequence, does not encode antibiotic resistance activity, and is not a reporter gene, and wherein said splice donor sequence is derived from a naturally-occurring eukaryotic splice donor sequence, said method comprising introducing said vector construct into said host cell under conditions such that said vector construct is integrated into the genome of said host cell.

270. (New) A method for making a eukaryotic host cell with a vector construct integrated into the genome of said cell, the vector construct comprising a promoter operably linked to an exon defined at the 3' end by an unpaired splice donor sequence, wherein said construct does not contain a poly-adenylation site operably linked to said promoter, wherein said construct does not contain a targeting sequence, and wherein said exon is derived from a naturally-occurring eukaryotic sequence, does not encode antibiotic resistance activity, and is not